



Atty. Docket No.: 1235(203284) PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Habener et al.	Examiner:	M.A. Belyavskyi
Serial No.:	09/963,875	Group Art Unit:	1644
Filed:	September 26, 2001		
Titled:	Stem Cell of the Islets of Langerhans and Their Use in Treating Diabetes Mellitus	Conf. No.:	9674

DECLARATION UNDER 37 CFR 1.132 BY ELIZABETH J. ABRAHAM, Ph.D

I declare:

1. I, Elizabeth J. Abraham hold a Ph.D degree from Kansas State University. I received my Ph.D degree in 1995. My current position is Senior Scientist at Viacell Inc. I have held this position since 2002. Previously, I held the position of Instructor at the Howard Hughes Medical Institute in the Massachusetts General Hospital, Harvard Medical School. I am co-inventor of the above-referenced patent application.
2. I have read the Office Action dated March 10, 2006, filed in the above-referenced patent application.
3. I understand that claims 39-41, 43, 74, 77-79, 81, 83 and 85-108 are rejected under 35 U.S.C. §103(a) for being allegedly unpatentable in view of Peck et al. (WO 97/15310) (referred to herein as the '310 reference).
4. I have read the '310 reference which has been cited during prosecution, and am familiar with the contents thereof.
5. The '310 reference relates to "the discovery that functional islets containing insulin-producing  $\beta$ -cells, as well as other islet cell types, can be grown in long-term cultures from pluripotent stem cells, which give rise to islet producing stem cells, IPSCs" (see page 8, lines 27-30). The '310 reference discloses at page 12, lines 21-23 that "IPSCs are a small population of cells derived from ductal epithelial cells (i.e., these cells are pancreas-derived but are not

differentiated islet cells).”

The ‘310 application teaches a method of growing IPSCs at page 13 line 29 through page 14, line 25 wherein it is stated that,

“ [t]he method of the subject invention involves making suspensions of cells, including stem cells, from the pancreas of a mammal. . . The cell suspensions are prepared using standard techniques. The cell suspension is then cultured in a nutrient medium that facilitates the growth of the IPSCs, while at the same time severely compromising the sustained growth of the differentiated or mature cells other than IPSCs. . . What is required for such media is that they have little or no glucose (less than about 1 mM) and low serum (less than about 0.5%). The high amino acid concentrations are preferably of amino acids known to be essential for the cells of the species being cultured, and provide a carbon source for the cultured cells. In addition, at least one rudimentary lipid precursor, preferably pyruvate, is provided. These conditions are so stressful to most differentiated cell types that they do not survive. Surprisingly, however, upon extended culture of cells from pancreatic tissue without re-feeding (about 3 weeks) IPSCs do survive and after extended culture, begin to proliferate.”

6. The instant application teaches one embodiment of the claimed method of isolating “a nestin-positive human pancreatic stem cell that is not a neural stem cell” in Example 1 (see page 48, line 15 through page 49, line 27).
7. Following the methods described in the instant application on page 48, line 17 through page 49, line 7, we have obtained an “isolated nestin-positive human pancreatic stem cell that is not an neural cell.” (see Figures 3 and 4 of the instant application).
8. Since the filing date of the instant application, other methods of obtaining “an isolated nestin-positive human pancreatic stem cell that is not a neural stem cell” have become known in the art.
9. There are substantial differences between the culture conditions described in the instant application for obtaining isolated nestin-positive human pancreatic stem cells that are not neural stem cells, and the culture conditions used in the ‘310 application to obtain the population of IPSCs described in the ‘310 application.

There are also substantial differences between the culture conditions of art-accepted methods for obtaining isolated nestin-positive human pancreatic stem cells that are not neural stem cells, and the culture conditions used in the '310 application to obtain the population of iPSCs described in the '310 application.

9. Exhibit A is a chart which compares the media, hormones and growth factors, and culture conditions used in the instant application (see Example 1, page 48, line 15 through page 49, line 27), or according to other art accepted methods for obtaining an isolated nestin-positive human pancreatic stem cell that is not a neural cell to the media, hormones and growth factors and culture conditions used in the '310 application (see pages 26 line 1, through page 28, line 2). As further detailed in the chart of Exhibit A, the '310 application does not teach culture conditions wherein immediately following trypsinization of islets, cells are cultured in media in the presence of glucose, high serum and growth factors.

10. We performed an experiment wherein replicate samples of islets were used to obtain isolated nestin-positive stem cells of the instant application according to the claimed methods, or according to the methods of isolating iPSCs described in the '310 reference. We note that although the '310 protocol uses Click's EHAA, we substituted DMEM due to the difficulty of obtaining a small amount of custom produced glucose-depleted or glucose free Click's media. One of skill in the art would accept that the use of DMEM would not affect the outcome of the experiment since the '310 reference teaches at page 14, lines 5 through 12 that [i]n a preferred embodiment, the nutrient medium is one which has a high concentration of amino acids. One such medium is known as Click's EHAA medium and is well known and readily available to those skilled in the art...Other equivalent nutrient media could be prepared and utilized by those skilled in the art. What is required for such media is that they have little or no glucose (less than about 1 mM) and low serum (less than about 0.5%)."

11. Upon receipt of human a islet (H1085) from Edmonton, Canada, the tissue (1000 Islet Equivalents (IEQs)) was trypsinized and 3.5 million cells were obtained.

12. According to the method described in the '310 application at pages 26, line 1, through

page 29, line 3, the cells were plated in six T-25 flasks in DMEM (no glucose and 0.25% FBS) without re-feeding for a period of three weeks. Within the first few days of culture there were very few attached cells. At the end of the three week period only cell debris was visible (Exhibit B). At four weeks the cultures were divided and re-fed with either Click's medium, supplemented with 0.5% FBS or RPMI supplemented with 10% FBS, bFGF and EGF (three flasks of each). Cultures were monitored for an additional two week period. No cells survived under either condition. Exhibit B presents representative photomicrographs taken 3.5 weeks after the initiation of the cultures. Virtually no live cells are present. The majority of what is detected is cell debris. All dead cell clumps and cell ghosts are non-adherent and appear necrotic. A-D represent four individual culture flasks. In panel A, dark necrotic cell clumps are indicated by the arrows. In panels B and C, cell debris and dead non-adherent cell clumps floating in suspension are indicated by the arrows. In panel D there is only cell debris with no live adherent cells in culture.

13. An isolated nestin-positive stem cell as required by the instant claims was obtained from a replicate sample of IEQs from the same islet isolate (H1085) used for the experiments according to the '310 protocol described above, according to the claimed methods. 1000 IEQs were washed in RPMI media and then plated in 10 cm<sup>2</sup> tissue culture plates in the following media: RPMI-1640 (11 mM Glucose) supplemented with 10% FBS and 20 ng/ml EGF. It is noted that although this embodiment of the claimed method of isolating a nestin-positive human pancreatic stem cell is not disclosed in detail in the instant application, this method was described in a post-filing publication from the inventor's laboratory (Abraham et al., 2002, *Endocrinology* 143:3152). The adherent cell population from these cultures was propagated and maintained in culture for over one month. Islets were plated in RPMI-1640 (11 mM glucose) supplemented with 10% FBS, antibiotics, HEPES buffer (1 mM) and 20 ng/ml EGF. Two weeks later, islet outgrowths were trypsinized and passaged regularly. Exhibit C is a representative photomicrograph of cells grown according to the claimed methods of the application, taken at five weeks post culture.

14. A comparison of cell proliferation and cell yield from each method is listed in Exhibit D.

15. The data presented herein demonstrate that an isolated nestin-positive human pancreatic stem cell that is not a neural cell, as required by claims 39-41, 43, 74, 77-79, 81, 83 and 85-108 of the instant application cannot be prepared according to the methods described in the '310 application.

I hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

8/22/06

Date

Elizabeth Jacob Abraham

Elizabeth J. Abraham



## EXHIBIT A

	Habener et al. (USSN 09/963,875)	'310 Reference (Peck et al.)
--	----------------------------------	------------------------------

### PRIMARY CULTURE CONDITIONS

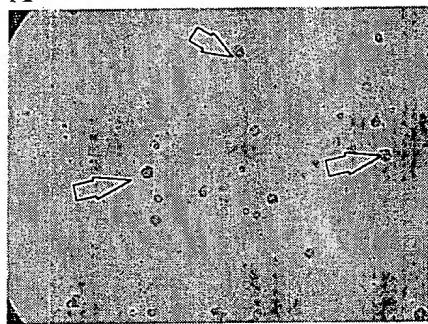
<b>Serum</b>	<b>10%</b>	<b>0.25%</b>
<b>Media</b>	<b>RPMI</b>	<b>Click's or comparable (DMEM used herein)</b>
<b>Glucose</b>	<b>11 mM</b>	<b>NONE</b>
<b>Refeeding</b>	<b>None for two weeks followed by weekly re-feeding</b>	<b>NONE (3 Weeks)</b>
<b>Growth Factors</b>	<b>None, EGF or EGF and bFGF</b>	<b>NONE</b>
<b>Matrix</b>	<b>None or Concanavalin A</b>	<b>NONE</b>

### SUBCULTURE CONDITIONS

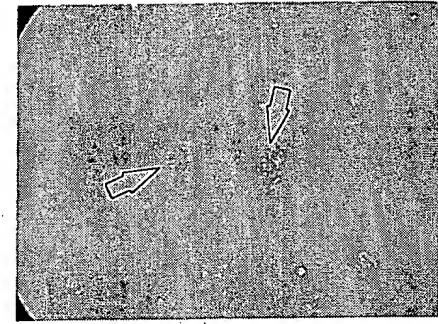
<b>Serum</b>	<b>10% FBS</b>	<b>0.5% FBS (Click's) or 10% (RPMI)</b>
<b>Media</b>	<b>RPMI</b>	<b>Click's or RPMI</b>
<b>Growth Factors</b>	<b>EGF or EGF and bFGF</b>	<b>None (Click's) or bFGF and EGF (RPMI)</b>

**EXHIBIT B**

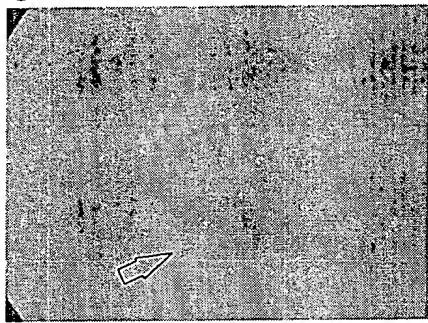
**A**



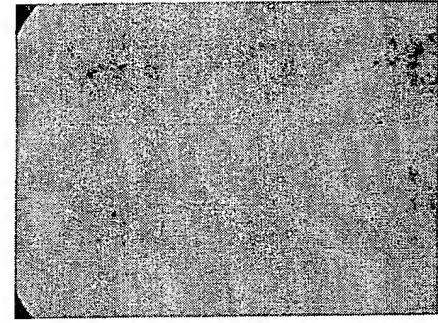
**B**



**C**

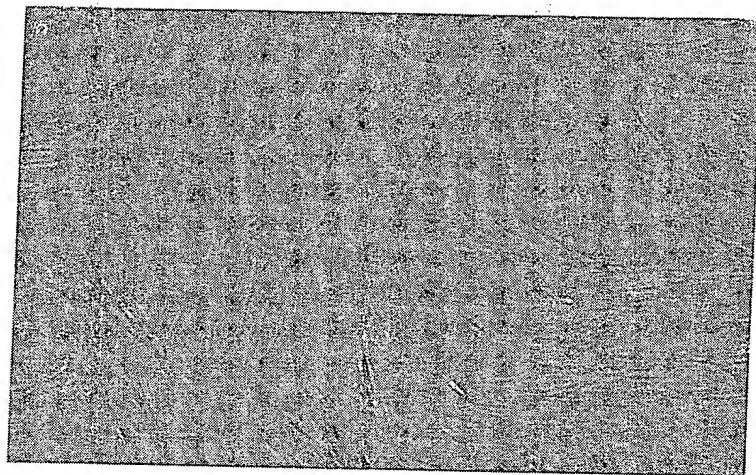


**D**



**BEST AVAILABLE COPY**

**EXHIBIT C**



**BEST AVAILABLE COPY**

## EXHIBIT D

Date	Fold increase		Cell yield	
	MGH	Peck	MGH	Peck
4/20/2006			1000 IEQ	1000 IEQ
4/24/2006			NA	Very sparse cells
5/2/2006			1.50E+06	NA
5/8/2006	5.29	NA	8.34E+06	NA
5/13/2006	3.24	NA	2.70E+07	NA
5/19/2006	4.1	NA	1.10E+08	Dead cells
5/25/2006	2.75	NA	3.04E+08	Cell debris